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# THE BACTERIA IN SCARLATINAL AND NORMAL THROATS.\*

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## INTRODUCTION.

It is a well established fact that streptococci are constantly found in great abundance on the tonsils of scarlet fever patients. This was shown very clearly by the investigations of Weaver, Baginsky and Sommerfeld, Charlton, Charbade, Booker, Taugl, Klein, Kurth and others. It is also well known that these organisms are frequently found on the tonsils of healthy persons, although not in such great abundance as on the tonsils of scarlet fever patients. Hilbert, Tunnicliff, Dungern, Schweighofer and Widal and Bezancon state that they found them in all normal throats which they examined. Others, however, did not find them so constantly. Black found them on only 30 per cent. of the normal tonsils which he examined. Netter found them in 5.5 per cent.; Kurth in 8 per cent.; Podbielsky in 2 per cent. and Dornberger in 45 per cent. of the normal throats examined by them. In most of these investigations no detailed study was made of the streptococci that were found. In some investigations glucose-broth tubes were inoculated with material from the tonsils, and if cocci were found in chains after incubation of the tubes it was concluded that streptococci were present. Apparently no further study was made of the cultures. In consideration of this fact it was thought worth while to take up this question again and make a more detailed study of the different strains of streptococci and diplococci which may be isolated from scarlatinal and normal throats.

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## METHOD OF STUDY.

Through the researches of Schottmüller<sup>1</sup> and of Rose<sup>2</sup> we know that pneumococci produce green colonies in blood-agar plates while *Streptococcus pyogenes* produces small grayish colonies which are surrounded by a perfectly clear area of hemolysis. Schottmüller also pointed out the fact that there are streptococci which produce green colonies in blood-agar plates, very similar to the pneumococcus colonies. In this study I have made use of these facts in isolating *Streptococcus pyogenes* from material obtained from the tonsils. A sterile cotton swab was rubbed against the tonsils in such a manner as to avoid the tongue as much as possible. The swab was then rinsed in 1-1.5 c.c. of sterile broth and the latter was used immediately to inoculate three or four blood-agar tubes, and the same number of litmus inulin-agar<sup>3</sup> tubes, which were poured into plates and incubated for 24 to 48 hours at 36° C. Blood-agar was prepared by melting plain agar tubes, cooling to 45° C. and adding .5 c.c. of sterile defibrinated rabbit blood or human blood to each tube.

After 24 hours' incubation it is possible to pick out the *Streptococcus pyogenes* colonies in the blood-agar plates, as they are small gray colonies which are surrounded by a perfectly clear area of hemolysis. It must be remembered, however, that diphtheria bacilli and pseudodiphtheria bacilli may produce hemolyzing colonies which may closely resemble the streptococcus colonies. Furthermore, I have occasionally found colonies of pneumococci and closely related organisms which produce considerable hemolysis and may therefore be mistaken for *Streptococcus pyogenes* colonies. The pneumococcus colonies are not easily recognized in these plates as there are always found in the throat many chain-forming cocci which form green colonies like the typical pneumococcus colonies. In all instances subcultures were made from 4 to 8 hemolyzing colonies and the same number of green colonies. These cultures were studied morphologically and culturally and their fermentative power was tested in Hiss<sup>4</sup> inulin serum-water medium or in a slightly modified medium which was prepared as follows: Dissolve 5 grams of NaCl, 20 grams of peptone (Witte) and

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1. Münch. med. Wochft., 1903, vol. L, p. 909.

2. Journal of Infectious Diseases, 1904, vol. i, p. 308.

3. Prepared as described in the Journal of Infectious Diseases, 1906, vol. iii, p. 183.

4. Jour. Exper. Med., 1905, vol. vi, p. 317.

20 grams of pure inulin in 1,000 c.c. of distilled water. Add 20 c.c. of a 5 per cent solution of pure litmus and tube, putting 2 c.c. of the mixture into each tube, and sterilize in the autoclave. After sterilization, add (with a sterile pipette) 2 c.c. of sterile heated ascites fluid<sup>5</sup> or preferably heated beef serum, to each tube and incubate for 24 hours before using. The beef serum was collected without taking any special precautions, but was diluted with an equal volume of water and passed through a large Berkefeld filter, drawn off into 50 to 100 c.c. tubes and heated to 65° C. for one-half hour on two successive days. This medium was preferred to Hiss' serum-water because it was found that some pneumococci and some streptococci which have been recently obtained from blood cultures do not grow well in the latter.

In the early part of this work no litmus inulin-agar plates were used but pneumococci were looked for among the green colonies in blood-agar plates. In those cases subcultures were made and studied from 10 to 12 green colonies. Organisms were classed as typical pneumococci only when they were found to be: 1. Gram-positive cocci which grew chiefly in pairs on blood-agar or serum-agar slants; and 2, which fermented inulin; and 3, produced green or slightly hemolyzing greenish colonies in blood-agar plates.

In the litmus inulin-agar plates most pneumococci produce red colonies which are easily recognized on the blue background, and as practically no other mouth bacteria produce red colonies in this medium<sup>6</sup> it is easy to isolate pneumococci from the mouth and throat with the aid of these plates. Subcultures were, however, always made from 2 or 3 red colonies, and the organisms positively identified. In no instance have I isolated an organism by this method which could not be classed as a pneumococcus, although some of these organisms form chains of considerable length in some liquid media and do not seem to have a well formed capsule.

#### RESULTS.

A total of 154 throat cultures were made and examined as follows: Normal, 51; scarlatina, 75; measles, 14; tonsillitis, 5; pneumonia, 5, and pharyngitis, 4. The

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5. Great care must be observed not to use ascites fluid which contains fermentable carbohydrates. Each lot must be tested with organisms that are known to have great fermentative powers and if acid is produced it must not be used.

6. See Journal of Infectious Diseases, 1906, vol. iii, p. 183.

findings differ very widely and must be considered under separate heads.

*Scarlatina*.—All the blood-agar plates inoculated with material from scarlatinal throats contained many *Streptococcus pyogenes* colonies and varying numbers of green colonies. This is shown by Figure 1 which is a photograph of one of these plates. The cases may be roughly divided into three great groups: 1. Those from which the plates contained mostly (60 to 95 per cent.) *Streptococcus pyogenes* colonies—31 cases. Two sets of plates contained only *Streptococcus pyogenes* colonies. 2. Those from which the plates contained about as many *Streptococcus pyogenes* colonies as green colonies—23 cases. 3. Those from which the plates contained fewer *Streptococcus pyogenes* colonies (15 to 40 per cent.) than green colonies—21 cases. As a rule, the *Streptococcus pyogenes* colonies greatly predominate over the other colonies when the inflammation of the throat is pronounced, and they rapidly decrease in number with the subsidence of the throat symptoms. When the throat symptoms are mild the proportion of *Streptococcus pyogenes* colonies to the other colonies is often very small. I have met with one exception to this rule in the case of a small girl who had a very bad throat, but the plates inoculated with material from it contained far more green colonies than *Streptococcus pyogenes* colonies. A large proportion of the green colonies in this set of plates were typical pneumococci which proved to be highly virulent for rabbits.

Pneumococci were not looked for in 4 cases, but were found in 64 of the 71 throats that were examined for them—91.4 per cent. Diphtheria bacilli were found four times and *B. mucosus* and *B. influenzae* once. *Staphylococcus aureus* and *citreus* and *Micrococcus tetragenus* were found occasionally, but these were probably contaminations from the tongue. Gram-negative diplococci which form small brownish colonies were found quite frequently. These were not always studied in detail, but some were identified as *Micrococcus catarrhalis*.

*Normal Throats*.—All blood-agar plates inoculated with material from normal throats contained many green and slightly hemolysing, often greenish, colonies. *Streptococcus pyogenes* colonies were found in 30 of 51 throats which were studied, but were never present in large numbers (Fig. 2), and were entirely absent in 21

cases. In only 5 sets of plates did I find more than 10 per cent. of *Streptococcus pyogenes* colonies, and in 18 sets there were only 1 per cent. or less of these colonies. Not infrequently one finds hemolyzing colonies in blood-agar plates which, on closer study, are found to be organisms that produce greenish colonies in subsequent plates. Great care must, therefore, be exercised in deciding on the presence or absence of *Streptococcus pyogenes* colonies. The small, slightly hemolyzing, often greenish, colonies of organisms other than *Streptococcus pyogenes* also are very puzzling and will be described more fully in a later publication. It may be mentioned here that sugar fermentation tests were frequently used in classifying these organisms. Pneumococci were found 45 times (90 per cent.), and diphtheria bacilli 3 times. Gram-negative diplococci and staphylococci were found frequently and *B. mucosus* and *B. influenza* once.

*Measles Throats.*—The findings in the throats of measles patients correspond closely to those of normal throats. *Streptococcus pyogenes* was found in 9 of 14 throats which were examined (65 per cent.) and pneumococci were found in 12 (85.7 per cent.).

*Tonsillitis.*—*Streptococcus pyogenes* colonies always predominated in these plates, but green colonies also were present. Pneumococci were found in 4 of the 5 cases examined, but were not specially looked for in the other case. In one case influenza bacilli were found in great abundance.

*Pharyngitis.*—Four cases of so-called pharyngitis were examined, but none of the blood-agar plates inoculated with material from these throats contained *Streptococcus pyogenes* colonies. All plates contained many green colonies and some contained small brownish colonies. The latter were chiefly Gram-negative cocci, but in one case they were influenza bacilli. Pneumococci were isolated from all of these cases. The finding of pneumococci in such a larger per cent. of throats of persons not afflicted with pneumonia is in agreement with the work of the Commission for the Investigation of Acute Respiratory Diseases of the Health Department of the city of New York.<sup>7</sup>

*Pneumonia.*—Three of the five sets of plates inoculated with material from the tonsils of pneumonia patients contained very few *Streptococcus pyogenes* colonies and the other two sets did not contain any. All

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7. Journal of Exper. Med., 1905, vol. vii, No 5.

blood-agar plates contained many green colonies and the litmus inulin-agar plates contained many red colonies. Typical pneumococci were easily isolated from all plates.

It will be noticed that green colonies were found in all blood-agar plates, except two sets, which were inoculated with material from scarlatinal throats. Only a small proportion of these colonies are pneumococci and the remainder form a large group of organisms between the typical pneumococci and streptococci. Some of these organisms seem to correspond to Schottmüller's *Strepto-*

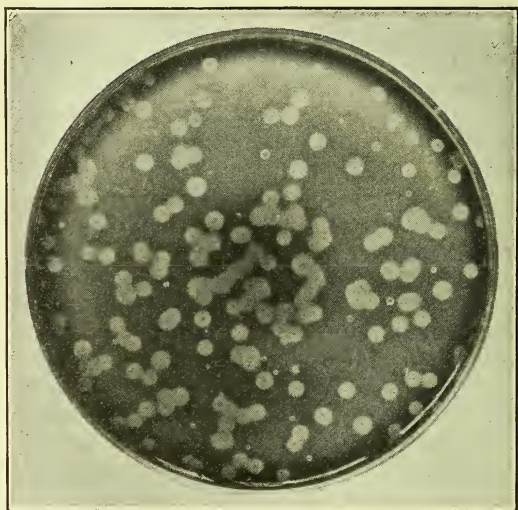


Fig. 1.—Photograph of a blood-agar plate inoculated with material from a scarlet fever tonsil. Many *Streptococcus pyogenes* colonies (hemolysing) and few small green colonies.

*coccus viridans*, but others are very closely related to the pneumococcus. The organisms of this large group have very little virulence for rabbits and seem to be normal inhabitants of the throat, especially the pharynx.<sup>8</sup>

#### VIRULENCE OF STREPTOCOCCI AND PNEUMOCOCCI ISOLATED FROM THROATS.

Most hemolysing streptococci and some pneumococci isolated from scarlatinal and normal throats have some virulence for rabbits, but *Streptococcus viridans* has

8. A more detailed description of these organisms and of different strains of *Streptococcus pyogenes* will be given in the Journal of Infectious Diseases.

practically no virulence for these animals. Thirty-one rabbits weighing from 900 to 1,200 grams were injected intraperitoneally with *Streptococcus pyogenes* cultures as follows: Four rabbits with a suspension of two forty-eight-hour blood-agar slant cultures of organisms isolated from normal throats. Three of these animals died and one remained well. Twelve rabbits with the same amount of suspension of organisms isolated from scarlatinal throats. Eight of these animals died and four remained well. Eight rabbits with a suspension of one blood-agar slant culture of organisms isolated from normal throats. Five of these animals died and three re-

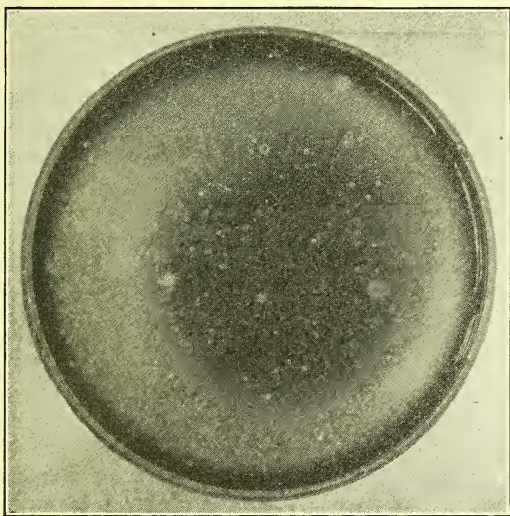


Fig. 2.—Photograph of a blood-agar plate inoculated with material from a normal tonsil. Many small green and slightly hemolyzing colonies and 5 *Streptococcus pyogenes* colonies.

mained well. Seven rabbits with a suspension of one blood-agar slant culture of organisms isolated from scarlatinal throats. One of these animals died and six remained well.

These results indicate that streptococci isolated from normal throats are slightly more virulent for animals than those isolated from scarlatinal throats. This is in perfect agreement with the results obtained by Hilbert<sup>9</sup> and Tunncliffe.<sup>10</sup>

9. Zeitschr. f. Hyg., 1899, vol. xxxi, p. 381.

10. Trans. Chicago Path. Soc., 1904, vol. vi, p. 175.

*Streptococcus viridans* has a lower virulence for rabbits than *Streptococcus pyogenes*, as has already been indicated. Nine rabbits weighing from 900 to 1,200 grams were injected into the peritoneum with two blood-agar slant cultures of different strains of these organisms, but only one of these animals died.

Ten rabbits were injected with two blood-agar slant cultures of different strains of pneumococci isolated from normal and scarlatinal throats. Four of these animals died and the other six remained well. Fourteen rabbits were then injected with larger doses, but only five of these died. There are no indications that pneumococci in scarlatinal throats are more virulent than those in normal throats, with the one exception referred to above.

#### CONCLUSIONS.

*Streptococcus pyogenes* is constantly found in great abundance on the tonsils of patients suffering from tonsillitis and scarlet fever before the inflammation of the throat has subsided. These organisms rapidly decrease in numbers with the subsidence of the throat symptoms.

*Streptococcus pyogenes* can not be considered a normal inhabitant of all healthy throats, although it was found in small numbers in 58 per cent. of the normal throats in this series.

Pneumococci of low virulence were found in 135 of this series of 154 throats.

A large group of organisms which lies between the typical *Streptococcus pyogenes* and pneumococcus was found in all normal throats and in nearly all diseased throats. These organisms have very little virulence for rabbits, and, as they are found in great abundance in practically all throats, they appear to be normal inhabitants of the throat.

*Streptococcus pyogenes* from normal throats appears to have a slightly greater virulence for rabbits than these organisms from scarlatinal throats.

I am indebted to Professor Hektoen for many suggestions and to Dr. W. L. Baum for the privilege of getting throat cultures from patients under his care at the Cook County Hospital.